Cytology/Biopsy/LEEP Cytologic-Histologic Correlation: Practical Considerations and Approaches

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Gynecologic Cytologic-Histologic Correlation

CLIA 88: “Laboratory comparison of clinical information, when available, with cytology reports and comparison of all gynecologic cytology reports with a interpretation of HSIL, adenocarcinoma, or other malignant neoplasms with the histopathology report, if available in the laboratory, and determination of the causes of any discrepancies.”

This requirement is generally referred to as cytologic-histologic correlation (CHC).

Cytologic-Histologic Correlation

- Approximately 45% and 20% of cytologic LSIL and HSIL, will not be verified on biopsy.
  - Clinical sampling error
  - Diagnostic imprecision.

Advice to pathologists:
- Avoid over calling minor histopathologic changes to achieve consistency with Cytology.
- ? Cytologic-Histologic correlation of over 80% or diagnosis of SIL that follow negative or equivocal colposcopic findings.

Crum, Crum, Rose and Peters, Chapter 13 of Crum, Crum, Lee, Diagnostic Gynecologic and Obstetric Pathology, 2011.

ACCME/Disclosure

Dr. Abdul-Karim has nothing to disclose
CAP: Quality Improvement

**Educational Group Microscopic Review of Select Cases**

**Optimize biopsies**
- Reorient tissue in block
- Obtain additional levels from the block
- Perform ancillary studies
- Record the presence or absence of the transformation zone in the report
- Develop laboratory policies for the number of routine serial sections and/or levels
- Coordinate with clinical colleagues to improve biopsy sampling
- Provide caregiver statistics on biopsy adequacy

**Monitor biopsy characteristics**
- Record the number of events where subsequent actions are necessary for discrepant biopsies
- Record negative cervical biopsies that:
  - Lack transformation zone
  - Are <2 mm
  - Are not associated with additional biopsies or endocervical curettage
  - Are poorly oriented
  - Require additional levels

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CAP: Quality Improvement

**Monitor Papanicolaou test characteristics:**
Record variables adversely affecting interpretation such as:
- Stain quality
- Thickness of preparation
- Obscuring factors
- Atrophic changes
- Did not have subsequently detected cells marked (screening variance)
- Demonstrate difficult patterns of detection

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Colposcopy

- Cervical colposcopy plays an essential role in the evaluation of women with abnormal cervical screening results
- Dissecting microscope with various magnification lenses (x5 – x25) providing an illuminated, magnified view of the cervix, vagina, and vulva
Colposcopy

Acetic acid solution (3-5%) is applied
- Dehydrates cells → squamous cells with relatively large or dense nuclei reflect light, appearing white.
  - Metaplastic cells
  - Dysplastic cells
  - Cells infected with human papilloma virus (HPV)

- Blood vessels and columnar cells are not affected → easier to visualize against the white background

- Abnormal vascular patterns:
  - Punctuation
  - Mosaicism
  - Atypical ("corkscrew," "hairpin") vessels

Images from Preinvasive Disease of the Cervix. Creasman, William T., MD, Clinical Gynecologic Oncology, Chapter 1, 1-50-46. Elsevier Science

The Accuracy of Colposcopic Biopsy

- “Colposcopy can easily determine the location and extent of 90% of cervical intraepithelial neoplasia (CIN) lesions.”

- The system of triaging cytological and histological abnormalities is frequently challenged, the actual “sensitivity” of the colposcopic exam is less frequently challenged.

- Colposcopic impression of HSIL identified only 56% of CIN2+ and the sensitivity for CIN 2+ of biopsy of colposcopically abnormal cervical epithelium is between 43.4% and 74.7%.

- The sensitivity of the first directed biopsy for CIN is around 52%.


Colposcopy at a Crossroads

- Consider a randomized trial of different approaches to diagnosis of patients presenting with HPV-DNA positivity and abnormal cytology. Issues of accuracy, cost, and comfort must be considered.
  - Additional biopsy from another part of the worst-looking lesion.
  - Additional biopsy of another abnormal area/s.
  - Random biopsy of quadrants that have no evident abnormalities.

- In the absence of new data, we personally do not recommend random biopsy specimens of normal appearing cervices.
**Agreement Between Colposcopically Directed Biopsies and Definitive Excisional Specimens (3 clinical trials)**

- There were significant associations in the agreement between biopsies and excisional specimen diagnoses:
  - Patients were stratified by age: Lesions in older patients may be larger.
  - Lesion size
  - Number of biopsies.
  - Presence of HPV 16/18.
  - Region (no difference).


**ALTS: Size of the CIN3 Lesion and Preceding Cytologic Interpretation**

- Aggressive follow-up of ASCUS and LSIL leads mainly to detection of CIN3 lesions that are smaller than those typically associated with invasion, especially when HPV testing is used.

- The identification of extensive CIN3 in a patient with ASC-US or LSIL is exceptional and should prompt a quality assurance review if practical to determine a possible role for sampling, screening, or interpretive errors.

- Cytology and colposcopy particularly underestimate the prevalence of small CIN3 lesions, which also have a slightly higher risk of a false negative HPV test.

**Size of CIN3 and Colposcopic Sensitivity**

- Using a logistic regression model; the most important predictor of increasing sensitivity of colposcopically directed biopsy was increasing size of CIN 3+.

- Suggests that cervical cytology of cancer or HSIL is a marker for large CIN 3+ rather than an independent predictor of higher sensitivity of colposcopically directed biopsy.


**Thin CIN: Difficult to Visualize**

- Average epithelial thickness (261 biopsies)

- Mean average epithelial thickness for 33 biopsies of CIN 2,3 from cervical quadrants with colposcopic impression of normal was less than that of 111 biopsies of CIN 2/CIN 3 from quadrants with colposcopic impressions of low, high, or cancer.

The inability of expert colposcopists to visualize some CIN 2/CIN 3 is associated with thinner epithelium.

Non-Correlating HSL and CIN1 or Less Biopsy Sampling Error

- HSIL Pap test (108) followed by cervical biopsy with or without subsequent cone/LEEP, there was a discordant cervical biopsy rate for HSIL of 43%.
- HSIL by Pap test followed up by cervical biopsy and subsequent cone/LEEP or repeat cervical biopsy, the proportion of women with negative colposcopic cervical biopsy and subsequent histology-proven HGCIN was 56%.
- These figures justify sampling error as a valid cause of non-correlation in women with HSIL followed up by cervical biopsy alone.

Number of Cervical Biopsies and Sensitivity of Colposcopy

- The overall sensitivity of the colposcopy procedure was similar across types of medical training.
- The sensitivity was significantly greater when the colposcopists took two or more biopsies instead of one.
- Independent of the severity of the colposcopic impression, the frequency with which colposcopists took two or more biopsies instead of one varied—
  - Nurse practitioners
  - General gynecologists
  - Gynecologic oncology fellows
  - Gynecologic oncologists.

Multiple Sampling Would Not Need to Increase the Cost of Pathology

- SPECIMEN SUBMITTED:
  A: Cervix, Biopsy 2:00
  B: Cervix, Biopsy 4:00
  C: Cervix, Biopsy 7:00
  D: Cervix, Biopsy 10:00
  E: Endocervical, Curettings

- SPECIMEN SUBMITTED:
  A: Cervix, Biopsy 3,6,9,12
  B: Endocervical, Curettings


Multiple Lesion-Directed Biopsies.

- 690 women: **Up to four directed biopsies**. A nondirected biopsy of a normal area was added if fewer than four directed biopsies.

- Sensitivities for detecting HSIL: 60.6% from 1 biopsy to 85.6% after 2 and to 95.6% after 3 biopsies.

- The highest increase in yield of HSIL: HG colposcopic impression, HSIL cytology, and HPV type 16 positivity. Only 2% of all HSILs diagnosed in the participants were detected by biopsies of normal-appearing transformation zone.

- Taking additional biopsies when multiple lesions are present should become the standard practice of colposcopic biopsy.

“Random Biopsies”?

- The incremental yield of CIN 3+ per colposcopy of the "random" biopsies decreased as the number of "random" biopsies increased, there was still a significant increase in yield of CIN 3+ per colposcopy with the fourth "random" biopsy.

- We advise up to 4 "random" biopsies at the SCJ in cervical quadrants without visible lesions: 25.7% of the CIN 3+ and 9.7% of the invasive cancers in this series were diagnosed by "random" biopsy.

- The importance of obtaining the cervical biopsies with a forceps that obtains small- lees painful (2 or 3 mm) biopsies cannot be overestimated.

Biopsy Tip Size of a “baby” and a Regular Tischler


- The natural history of the smaller CIN 2 diagnosed by "random" biopsy may have a higher rate of spontaneous resolution (Smaller, involved fewer quadrants and were lower grade) than CIN 2 diagnosed by colpo-directed biopsy.
**ATHENA trial: Random Biopsy and Negative Colposcopy: Genotyping**

- The random biopsy diagnosed 20.9% and 18.9% of the total CIN2+ or CIN3+.
- For HPV 16 or 18, the absolute risk for detection of CIN 2+ non random biopsy in the overall population was 13.1% and 8.2% for CIN 3+. By contrast, the absolute risk for 12 other high-risk HPV+ women was 3.5% and 1.7% for CIN 2+ and CIN 3+
- Supports performing a random biopsy in women undergoing colposcopy without visible lesions, particularly in those positive for HPV 16 or 18.

**Distribution of Neoplasia Arising on the Cervix**

- Sampling the anterior and posterior cervical lips in hysterectomy specimens provides the best opportunity for detecting unsuspected cervical intraepithelial neoplasia (CIN)?
- CIN affected one or other cervical lip in all 100 cones studied and involved the midline positions (12 and 6 o'clock) in 94. The lateral edges of the cervical canal were also involved in 38 cases.
- CIN is more likely to be identified on the anterior and posterior lips than on the lateral aspect of the cervical os. The findings support the continuation of the established practice of taking blocks from the midline in these areas.

**Obtaining Additional Tissue Levels**

- Complete step sectioning of paraffin blocks was undertaken on 111 non-correlating biopsy specimens from 95 patients and selected slides were reviewed for the presence of SIL.
- 27 biopsies (24.3%) demonstrated the presence of a SIL in deeper levels.
- Additional tissue levels are a productive way of confirming SILs, allows a refined selection of negative cervical biopsies most likely to reveal an SIL on review of deeper levels.

**Additional Deeper Levels**
Additional Deeper Levels: How many?

- 600 consecutive biopsies from 404 patients were reviewed.
- If sectioning were limited to 3 levels, 17.5% (105/600) of all dysplastic lesions would have been missed, including 19.6% (100/511) of CIN 1 and 5.6% (5/89) of CIN 2-3.
- Not more than 3 levels are routinely evaluated in most laboratories.
- Using our clinical efficacy standard, when no pathologic findings are initially identified in a colposcopic-directed biopsy, at least 5 levels (a priori or in recuts) are required to ensure a 100% diagnostic accuracy for CIN 2-3.

Prior HSIL/ASC-H Pap: Additional Deeper Levels x5

ALTS: Inter-observer Reproducibility of Cervical Cytologic and Histologic Interpretations

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<thead>
<tr>
<th>Technique</th>
<th>% Agreement</th>
<th>Kappa</th>
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<tbody>
<tr>
<td>Thin-layer</td>
<td>62.0</td>
<td>0.46</td>
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<tr>
<td>Colpo Bx</td>
<td>62.0</td>
<td>0.46</td>
</tr>
<tr>
<td>LEEP</td>
<td>69.9</td>
<td>0.49</td>
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Interpretive variability is substantial for all types of cervical specimens. Histopathology of cervical biopsies is not more reproducible than monolayer cytology, and even the interpretation of LEEP results is variable. Given the degree of irreproducibility that exists among well-trained pathologists, realistic performance expectations should guide use of their interpretations.


Agreement Between Colposcopically Directed Biopsies and Definitive Excisional Specimens (3 clinical trials)

• 737 women (16–45y).

• The overall agreement between the biopsies and the definitive therapy diagnoses was 42%. The overall underestimation of CIN2-3/AIS and CIN3/AIS was 26 and 42%, respectively.

• Accuracy improved when CIN2 and CIN3/AIS were grouped together: HG

• Colposcopy functioned well when allowed a one-degree difference between the biopsy and the surgical histologic interpretations, as done in clinical practice: 92% overall agreement for CIN2-3/AIS

ALTS: Inter-observer Viability of CIN1

• An interpretation of CIN 1 by the CC was corroborated by the QC group in only 42.6% of 887 biopsies.

• Equal proportion of originally diagnosed CIN 1 biopsies (41.0%) were interpreted as negative by the QC group.

• Biopsies diagnosed as negative or CIN2+ had 90.8% and 76.9% concordance.

• The poor reproducibility of the histologic diagnosis of CIN 1, as well as the uncertain biological potential of lesions that are classified based on their histologic appearance as CIN 1, makes management of these women problematic.

Pap: ASC-US/HPV+
Biopsy: LSIL (CIN1)
ALTS: Biopsy Negative or LSIL- 2 year Follow Up

- 897 cases of LSIL and 1193 cases of ASCUS HPV+ CIN1 or less.
- Cumulative risk of CIN 2-3 was equivalent for LSIL (27.6%) and ASCUS HPV+(26.7%).
- After excluding the women with a diagnosis of CIN2/3 at initial colposcopy and biopsy (17.9%), the remaining were at nearly identical risk for subsequent CIN 2/3 regardless of initial colposcopy result.
- For women who have CIN1 or less on colposcopy and biopsy, the risk for subsequent CIN 2/3 is approximately 12% over 2 years. This risk does not vary meaningfully by initial distinction of histologic CIN grade 1 from negative colposcopy and biopsy.

CIN3: ALTS-Prevalent vs Incident. Within 2 Years of a Minor Cytologic Abnormality.

- 17 (2.8%) of 613 CIN 3 diagnosed during the 2-year duration were incident CIN 3 following an incident HPV infection that persisted until the CIN 3 diagnosis was made.
- Using prevalent HG cytology as a marker of prevalent CIN 3, estimated that another approximately 23% of CIN 3 cases were incident CIN 3 following a prevalently detected HPV infection that persisted until the CIN 3 diagnosis was made.
- Most CIN 3 cases diagnosed within the 2-year time frame were prevalent cases, and most incident CIN 3 cases followed a prevalently detected HPV infection.

Longitudinal Evaluation of Inter and Intra-observer Agreement of CIN: 4 Experts

- QC slides (185) with CIN diagnoses
- Panelist pair: CIN+ versus non-CIN+ or CIN 2/3+ versus non-CIN 2/3+
- Higher diagnostic agreement indicate that a well-established panel of experienced pathologists can achieve very high [kappa] values for both interobserver and intraobserver agreement.

The Interpretive Variability of Cervical Biopsies

- 6272 biopsies, extrapolated to 21,297 biopsies read by CP
- Panel agreement with the community diagnosis:
  - CIN1: 38.2% (Fewer CIN1 and more negative diagnoses in the P review but similar proportions of CIN2/3).
  - CIN2: 38.0% (Significant variability in the CP and P diagnoses)
  - CIN3: 68.0%
  - HPV16 and hr-HPV positivity increased with disease severity, but P review did not improve the correlation of higher-grade disease with these objective measures.
- New biomarkers are needed to more accurately stratify precursor lesions according to their malignant potential.
- The use of antecedent cytology results influenced the diagnostic process in a limited way, but more definitive markers are needed.
**LAST/P16: Recommendation No. 4a**

- **Special Circumstance.**—p16 IHC is recommended as an adjunct to morphologic assessment for biopsy specimens interpreted as ≤–IN 1 that are at high risk for missed high-grade disease, which is defined as a prior cytologic interpretation of HSIL, ASC-H, ASC-US/HPV-16+, or AGC (NOS).

- Any identified p16-positive area must meet H&E morphologic criteria for a high-grade lesion to be reinterpreted as such.
### Biopsy CIN3 and Negative LEEP

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<tr>
<th>Biopsy CIN3</th>
<th>Negative LEEP</th>
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<tr>
<td><img src="image1.jpg" alt="Biopsy CIN3 Image" /></td>
<td><img src="image2.jpg" alt="Negative LEEP Image" /></td>
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### LEEP: Role of Time Between Biopsy and Excision

- 391 women with biopsy CIN 2-3, 26.9% had LEEP specimens with CIN ≤ 1 histologies. The likelihood of a CIN ≤ 1 LEEP specimen increases for greater biopsy-LEEP intervals.
- The rate of spontaneous histologic regression (defined as CIN ≤ 1 at resection) was 26.9%.
- These low-grade lesions were more common in LEEP specimens from young women with CIN 2 at biopsy, and who underwent LEEP later after the initial biopsy.

### Do Colposcopically Directed Biopsy and ECC Serve to Induce Regression of Cervical Intraepithelial Neoplasia?

- 555 patients with excision for CIN2+.
- Median interval from colposcopy to excision was 48 days.
  - Neither demographics nor colposcopic findings influenced the probability of regression.
- Regression was less likely with longer latency from colposcopy to excision:
  - Emergence and documentation of persistent occult neoplasia.
  - Lesion is incompletely excised.
  - Minute occult lesions escape discovery
  - Biopsy may not affect immune recognition allowing the natural history to unfold, and the neoplasia is at risk of progression during this latency interval.

### Endocervical Curettings (ECC)

- ECC: Appears to be more associated with regression. This is especially true when there is no visible lesion at the time of colposcopy, and curettage may theoretically remove an isolated occult endocervical lesion completely.
- Serves to excavate an area of the endocervix large enough to remove neoplasia completely, or whether it causes an inflammatory reaction leading to increased immune recognition.
- Do not support the routine ECC in all patients.
- However, among postmenopausal women, those with HSIL, large lesions, or unsatisfactory examination results, ECC remains crucial.
- ECC detected 5.4% - 9.3% of CIN2+ cases missed by biopsy.
ECC: CIN2/3- p16

Do Colposcopically Directed Biopsy and Endocervical Curettage Serve to Induce Regression of Cervical Intraepithelial Neoplasia?

- Normal excisional biopsy results do not necessarily indicate that the procedure was aggressive or unnecessary.
- In some cases, the results can indicate that colposcopic biopsy practices served as treatment.
- The study does not support the theory that colposcopic biopsy performed by experienced colposcopists in a routine practice setting excises lesions sufficiently to remove all intraepithelial neoplasia or to stimulate an immune response sufficient to induce regression.
- Large lesions were more likely to be high grade, which may explain why a punch biopsy or curettage does not sufficiently remove the lesion and induce regression by the time of excision.

Examining Sources of Diagnostic Error Leading to Cervical Cone Biopsies with No Evidence of Dysplasia

- 53 cone biopsies initially reported as negative for dysplasia or malignancy (17% of all cone biopsy specimens).
- Each negative cone biopsy specimen was examined with at least 3 deeper levels. If dysplasia not identified on deeper levels, p16 stain was performed on the most atypical level.
- Additional review by 3 pathologists for consensus diagnosis
  - 4 cases (7.5%) were identified by additional level sections (two-dimensional sampling of a three-dimensional specimen)
  - 7 cases (13.2%) were identified by additional levels and p16
  - 3 cases (5.7%) were found by consensus review

Remaining 39 cases that remained negative with additional workup:
- 15 cases (28.3%) were attributed to overinterpretations on pre-surgical specimens.
- 24 patients HSIL confirmed: 6 (11.3% of the total) had confirmed dysplasia or carcinoma on follow up.

The overall false-negative rate for cone biopsy specimens, when the fourth category of undersampling was added, was 21%.
LEEP: Initial Level Negative; Deeper HSIL

LEEP: Initial Level "Atypia"; P16 positive; Deeper HSIL
Cytologic-Histologic Correlation

Colposcopy:
– Patient’s age: Lesions in older patients may be larger.
– Lesion size
– Number of biopsies/Random/ECC
– Presence of human papillomavirus (HPV)16/18.

Pathology:
• Deeper levels
• Inter-observer reproducibility
• Consensus agreement
• P16 staining